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THE CONSEQUENCES OF SUBSEQUENT EXPOSURES OF MILD AND MODERATE HYPOXIA ON THE FLIGHT PROFILE

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Abstract

Previous studies of hypoxia have largely examined different altitudes in isolation. Pilots, however, receive two sequential exposures during in-flight hypoxic emergencies (IFHEs): the initial exposure at altitude, followed by a second mild exposure after descending and removing the breathing mask. Conventional wisdom holds that performance recovers with blood oxygen saturation and that exposure to mild hypoxia is safe, but recent studies have challenged these assumptions. This study examined the possibility that the effects of moderate hypoxia may linger to overlap with the effects of mild hypoxia to increase performance deficits during sequential exposures such as those experienced by pilots during an IFHE. Participants performed a simulated flight task and secondary task while being exposed to normobaric hypoxia via the ROBD-2. We hypothesized that performance during exposure to mild hypoxia would be worse when preceded by a moderate hypoxic exposure than when mild hypoxia was experienced in isolation. Our hypothesis was partially supported: performance during exposure to 10,000 foot-equivalent altitude was worse when preceded by exposure to 25,000 foot-equivalent altitude, but we believe that this is most likely due to a failure to recover from the original moderate exposure rather than an additive effect between the exposures. Even so, our findings suggest that pilot impairment following an IFHE may be worse than previously believed.

Introduction

Background

Most research examining the effects of hypoxia on performance has studied single exposures in isolation. However, this may not accurately reflect the exposures received by pilots during an in-flight hypoxic emergency. Conventional wisdom assumes that hypoxia-related performance deficits disappear following a return to normal blood oxygen saturation (SpO_2), and that mild hypoxia does not impair performance. Based on these assumptions, pilots experiencing hypoxia breathe 100% oxygen to return to normal SpO_2 while descending to a cabin altitude of 10,000 feet, at which point they remove the flight mask and breathe cabin air. As a result of this procedure, pilots actually receive sequential exposures during an emergency: moderate hypoxia during the emergency itself, followed by mild hypoxia upon removing the flight mask. The present study examined whether the effects of sequential exposure to moderate and mild hypoxia are different than either exposure alone. For the purposes of this paper, "moderate" hypoxia will refer to a hypoxic exposure that is sufficient to cause notable symptoms and performance deficits, but not immediate incapacitation. "Mild" hypoxia will refer to a hypoxic exposure insufficient to cause subjective discomfort.

Recent experimental data has challenged the assumptions underlying current emergency procedures, calling the efficacy of these procedures into question. Performance may not recover immediately after a hypoxic episode even when SpO_2 returns to normal (Phillips et al., 2009; Phillips, Simmons, & Horning, 2012), and altitudes previously believed to be safe can cause performance deficits (Petrassi, Hodkinson, Walters, & Gaydos, 2012; Legg et al., 2012; Legg et al., 2014). Potential lingering effects of moderate hypoxia may therefore overlap with the effects

of mild hypoxia after the pilot descends and removes the breathing mask, but the possible effects of such combined exposures have not been explored.

The effects of moderate hypoxia

Exposure to moderate hypoxia leads to deficits in several flight-related cognitive functions including visual processing, attention, reaction time, and motor control (Artino, Folga, & Swan, 2006; Fowler, Banner, & Pogue, 1993; Fowler, Taylor, & Porlier, 1987; Fowler, White, Wright, & Ackles, 1982). Accordingly, moderate hypoxia has been shown to affect pilots' ability to perform even simple tasks such as maintain a prescribed airspeed and altitude during simulated flight (Temme, Still, & Acromite, 2010). Further, although SpO_2 typically returns to baseline within one minute of breathing normal oxygen levels, performance on certain tasks may not return to baseline levels for several hours. Some tasks such as contrast sensitivity recover fairly rapidly (Phillips, Simmons, & Horning, 2012). Other tasks such as the Flanker Arrow Task or simple and choice reaction time recover much more slowly. The Flanker Arrow Task (designed to measure reaction time and attention in the presence of distracting stimuli) demonstrated impairment throughout a 10 minute recovery period after exposure to 20,000 feet simulated altitude (Phillips et al., 2009), and simple and choice reaction time were impaired up to 24 hours after simulated exposure to 18,000 feet (Phillips, Simmons, & Horning, 2012).

The effects of mild hypoxia

Altitudes below 10,000 feet are generally not considered to cause performance deficits, but research findings indicate that this assumption is not true in all circumstances. Exposure to 10,000 feet for as little as 15 minutes can cause color vision deficits under the lighting conditions encountered during night flying (Connolly, Barbur, Hosking, & Moorhead, 2008). Exposure to

10,000 feet also increases procedural errors during simulated flight, particularly during descent and landing (Nesthus, Rush, & Wreggit, 1997). Finally, Legg and colleagues (2012; 2014) indicate that performance on difficult cognitive tasks such as complex logical reasoning or demanding memory tasks may begin to show marginal impairment after exposure to altitudes as low as 8,000 feet. Although the deficits associated with mild hypoxia are relatively minor, even subtle impairments in vision, procedural execution, reasoning, or memory can increase the risk of a mishap following an in-flight hypoxic event.

The present study

The separate effects of mild or moderate hypoxia are each sufficient to compromise flight safety. Performance deficits may not disappear upon return to normal SpO_2, potentially leading to a situation where the effects of moderate hypoxia linger to interact with the effects of mild hypoxia during a normal response to a hypoxic emergency. The possibility that the effects of mild hypoxia may be exacerbated by prior exposure to moderate hypoxia is especially concerning when coupled with the fact that the most pronounced effects of mild hypoxia affect tasks associated with the descent and landing phase of flight. Given that descent and landing are a pilot's primary objectives after initiating emergency procedures, pilots are exposed to mild hypoxia precisely when its effects are most serious. The risk of pilot error following a hypoxic event may therefore be greater than is currently believed.

The present study evaluated whether sequential exposures to moderate and mild hypoxia such as those experienced by pilots in a hypoxic emergency lead to greater performance deficits or increased recovery time compared to a single altitude exposure. Due to the possibility of a "hangover" effect from a moderate exposure interacting with the effects of a mild exposure, we hypothesized that successive exposure to moderate and mild hypoxia as encountered when

following current emergency procedures would worsen performance deficits and/or impair recovery compared to either exposure alone. We were particularly concerned that performance at the traditional "safe" altitude of 10,000 feet would be worse following a moderate exposure than when 10,000 feet is experienced in isolation.

Method

Participants

A total of 21 active duty military personnel assigned to Wright-Patterson Air Force Base, OH completed this study. Participants included 20 males and 1 female ranging in age from 22 to 37. Participants were screened prior to participation to rule out any medical conditions or lifestyle issues that may have compromised safety or confounded the results (e.g., asthma, anemia, sickle cell trait, history of fainting, tobacco use, excessive alcohol use, etc.; the full list of screening criteria is included in Appendix 1). None of the participants were licensed pilots, but some did report an interest in flying and prior experience using flight simulators. Fourteen participants reported prior experience with hypoxia.

Apparatus

Reduced Oxygen Breathing Device (ROBD-2)

Participants were exposed to normobaric hypoxia via the Reduced Oxygen Breathing Device (ROBD-2; Environics). The ROBD-2 is a gas blending device that uses thermal mass flow controllers to deliver mixtures of compressed breathing air, nitrogen, and oxygen to simulate altitudes between ground level and 34,000 feet without altering the barometric pressure experienced by participants. Gas mixtures were delivered through a standard aviation mask attached to a flight helmet via bayonet clips.

Flight simulator

Participants performed tasks in a fixed-based flight simulator operated via X-Plane software emulating a T-6 Texan. The flight instruments were displayed on a 26 inch diagonal ELO monitor, while the outside-the-cockpit view was displayed on a 60 inch diagonal Samsung LED High Definition TV, providing an 87º wide by 49º high field of view. A FitPC3Pro drove the outside the window scene graphics. Participants sat in an open cockpit on a SPARCO seat adjustable for height and seat back angle. Control inputs were made using a Thrustmaster Cougar joystick and Thrustmaster Warthog throttle.

Physiological monitoring

SpO_2 was monitored using a standard pulse oximeter (Model 3900P, Datex Ohmeda Corp.) placed on the index finger of the left hand. The pulse oximeter on the finger was worn by every participant for every visit and served as the primary monitoring tool during each exposure.

As part of a concurrent effort examining the validity of various additional physiological sensors, participants wore one of several different sensors on their foreheads during each visit. The sensors are described here for completeness, but will not be discussed further in this report. The first set of sensors was a Near Infrared Spectroscopy sensor (NIRS; PocketNIRS, Dynasense) worn on the left side of the forehead together with a previously validated NIRS sensor (INVOS Cerebral Oximeter, Somanetics) on the right side of the forehead. The second sensor suite was a reflectance pulse oximeter and NIRS sensor integrated into a single headband (Canary, Elbit). Finally, a separate pulse oximeter was also evaluated (Nellcor PM100N Bedside SpO_2 Patient Monitoring System, Covidien). The Covidien sensor was attached at the left side of the forehead and secured with an elastic headband. Participants wore only one type of sensor on the forehead during each visit. Although an effort was made to keep the forehead sensor worn by

each participant consistent across visits, many participants wore multiple sensors over the course of the study due to changes in sensor availability during the study.

Performance tasks

Participants performed two tasks during the exposure profile: a flight task and a time estimation task. Participants were not instructed to prioritize one task over the other. The primary flight task consisted of maintaining a straight and level course on a heading of 90° at an altitude of 12,000 feet and an airspeed of 150 knots. Participants were instructed that all three parameters would count equally toward their performance score. Participants used only the control stick and throttle to fly the aircraft – all other controls and cockpit switches were disabled. The aircraft was untrimmed and a steady quartering wind was blowing from 45° at five knots. Participants flew over a simulation of the terrain around Fallon Naval Air Station, Nevada, with clear weather. Similar straight-and-level flight tasks in simulators have proven to be sensitive to the effects of hypoxia (Temme, Still, & Acromite, 2010).

Participants also performed a secondary task consisting of estimating 10 second intervals. While flying, participants received a prompt to "Begin counting 10 seconds now" displayed on the outside-the-cockpit monitor as well as broadcast through speakers mounted to the simulator. Prompts were randomly timed to occur between 20 and 30 seconds apart. After each prompt, participants started the timer by pressing a button on the control stick. When the participant estimated that 10 seconds had elapsed, the participant pressed the same button again to stop the timer. Upon activation/deactivation of the timer, the perimeter of the outside-the-cockpit monitor flashed red to acknowledge the button press. Other than this indication that the timer had been successfully activated/deactivated, participants did not receive feedback regarding the time estimation task. Both length and variability of time estimation have been shown to be sensitive to

workload and difficulty manipulations in flight simulators (Bortulussi, Hart, & Shively, 1989; Bortolussi, Kantowitz, & Hart, 1986; Casali & Wierwille, 1983).

Study design and procedure

Design

Hypoxia exposures followed a 2x2 within subjects single blind design (Figure 1). Each exposure profile consisted of two altitudes. Altitude Equivalent 1 was either ground level[1] or 25,000 feet normobaric equivalent. Altitude Equivalent 2 was either ground level or 10,000 feet normobaric equivalent. For all flight profiles, participants breathed ground level air for five minutes (Segment 1; S1), followed by Altitude Equivalent 1 for five minutes (Segment 2; S2), another five minutes of ground level air (Segment 3: S3), Altitude Equivalent 2 for 30 minutes (Segment 4; S4), and a final five minutes of ground level air (Segment 5; S5). Participants were blinded regarding which flight profile they experienced on any given visit. The order of the flight profiles was counterbalanced across participants using a Latin Square design. See Figure 2 for a depiction of the flight profiles and the segments of each.

		Altitude Equivalent 2	
		0	10k
Altitude Equivalent 1	0	A	B
	25k	C	D

Figure 1: Experimental design for the present study

S1 allowed the participant to acclimate to breathing through the ROBD-2 and wearing the equipment prior to hypoxic exposure. S3 allowed the participant to return to normal SpO_2 between simulated altitudes (thus separating the two exposures and reducing attrition due to low SpO_2 or participant withdrawal). S5 allowed the researchers to verify that the participant returned to normal saturation levels prior to the end of the flight profile. The exposure times for Altitudes

[1] The testing laboratory at the Naval Medical Research Unit - Dayton is located approximately 823 feet above sea level.

1 and 2 were within the limits listed in the Time of Useful Consciousness table (DeHart, 1985), and are reasonable estimates of how long exposure to each altitude may last in an aircraft as the pilot must first recognize hypoxia (Altitude Equivalent 1), descend, and then fly to an airfield to land after removing the flight mask (Altitude Equivalent 2).

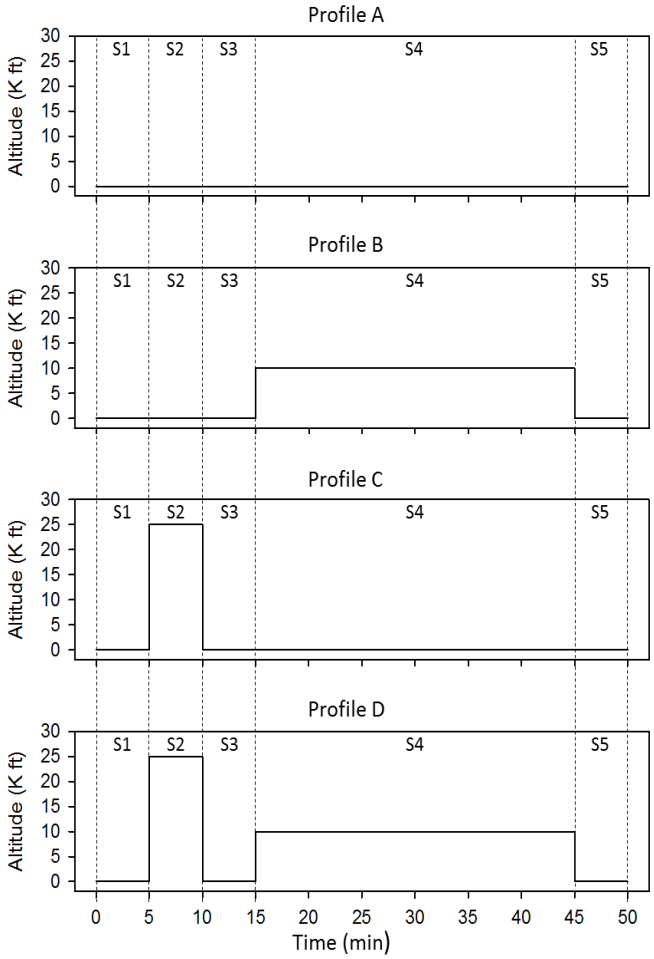

Figure 2: Exposure profiles for Conditions A, B, C, and D (see Figure 1 above), as well as the segments used for analysis.

Arrival

Participants reported to the Naval Medical Research Unit – Dayton (NAMRU-D) on four separate occasions, experiencing a different exposure profile each visit. Visits were scheduled a minimum of 48 hours apart in order to ensure that the effects of hypoxia dissipated completely

between visits. Upon arrival for their first visit to the laboratory, the participant was escorted to a wet lab where the study was explained and the participant had an opportunity to read the informed consent document and ask questions. After giving informed consent, participants completed a brief questionnaire to confirm compliance with study requirements (Appendix 2), followed by a blood pressure check and a blood draw to ensure normal levels of hematocrit and hemoglobin (blood draw results remained valid for 72 hours; participants with prior medical clearance to become hypoxic due to routine hypobaric chamber or other exposures were exempted from the blood draws). Female participants were given a urine pregnancy test each visit to rule out pregnancy prior to any exposure. Following the informed consent, questionnaire, and physiological checks, participants were brought to the hypoxia lab to be fitted for a flight helmet and flight mask. After equipment fitting, participants were trained on the performance tasks and allowed to practice until they felt comfortable (approximately five minutes for most participants). Visits after the first visit followed the same procedure except for the informed consent and equipment fitting.

Hypoxia exposure

Once participants indicated that they were comfortable with the flight task, the simulator was reset and the participant donned the helmet, flight mask, and physiological monitoring equipment. At this point the experimenters reminded the participant about the timing of the flight profile, gave the participant a five second countdown, and began the exposure. Participants performed both the flight task and the time estimation task for the entire duration of the exposure profile. In addition to the physiological sensors, participants were monitored via closed-circuit video as well as audio communication with the experimenters. Exposure to each altitude was terminated and the participant was advanced to the next ground level portion of the profile after

the time limit was reached, if the participant's SpO_2 dropped to 55% at the finger or 60% at the head, if the participant became nonresponsive to verbal prompts, or if the participant requested to be brought back to ground level.

Recovery

Upon completion of the flight profile, the experimenters disconnected the physiological monitoring equipment and escorted the participant to a break room where entertainment and snacks were provided. One hour and again two hours after the end of the exposure, the participant was reconnected to the monitoring equipment and completed a five minute flight while breathing ground level oxygen concentrations through the ROBD-2 (Recovery 1 and 2, respectively).

Analysis and Results

Data collection and processing

ROBD-2 oxygen concentration and pulse-oximeter (PO) data were collected in LabView (v8.2, National Instruments). X-Plane output and cognitive performance data were collected via a custom plugin and instructor operating station (IOS) written in the C# and C++ languages. All data processing, including time line-up and calculation of physiological and outcome measure statistics, was performed in MATLAB (MathWorks, Inc.). Repeated measures ANOVAs were performed in SPSS (IBM).

Outcome measures

Flight task

The outcome measure for the flight task was normalized root mean square error (NRMSE). For each flight parameter (i.e., heading, airspeed, and altitude), NRMSE was computed as:

$$NRMSE = \sqrt{\frac{\Sigma(actual\ value - target\ value)^2}{n}} \times \frac{1}{target\ value}$$

where *n* is the number of data points. NRMSE was then summed across each parameter within a given segment of the flight profile to derive a single value accounting for total error in airspeed, altitude, and heading during each segment of the flight profile (Total NRMSE). Total NRMSE will be hereafter referred to as flight-sim error (FSE).

Previous work in our lab demonstrated that a flight simulator performance metric that essentially mirrors FSE (flight simulator lapses) was very nearly normally distributed; we therefore had reason to expect changes in FSE to be normally distributed. Further, there is precedent for analyzing flight simulator performance using NRMSE and ANOVA (e.g., Krueger, Armstrong, & Cisco, 1985; Previc et al., 2009; Smith & Caldwell, 2004).

Time estimation task

Outcome measures for the time estimation task included lapses per minute (LPM) and standard deviation in the time estimates (TSD).[2] Lapses were defined as any response pattern that did not match the prescribed order of "prompt – start timer – stop timer". For instance, if the participant failed to start the timer when prompted or started the timer without stopping it before the next prompt the trial was scored as a lapse. Responses were also scored as a lapse if the participant started the timer, stopped the timer, and then pressed the response button again before the next prompt. This decision was made on the assumption that the participant had likely become confused as to whether they had already started or stopped the timer. In addition, because extremely low time estimates were observed to occur in conjunction with multiple failed

[2] Accuracy of the time estimates was not used as an outcome measure because many participants tended to consistently over- or underestimate the 10s target. A variance criterion was therefore considered a better gauge of task performance across the exposures.

starts/stops (indicating confusion), a trial was considered a lapse if the estimate was shorter than three SD below the participant's mean estimate for the profile. Lapses were standardized by total timespan of segments in minutes because the duration of S2 was often shorter in conditions C and D compared to conditions A and B.

Physiological Measures

The primary physiological measures of interest were SpO_2 and heart rate (HR) as measured by the Datex-Ohmeda PO at the finger. A low pass Butterworth filter with a cutoff frequency of 0.08 Hz was applied to the raw HR data to reduce variance attributable to the 3s averaging mode of the PO, thus stabilizing HR extremes. HR measures were calculated from filtered HR. Measures compared included minimum SpO_2 (SpO_2 Min), average SpO_2 (SpO_2 Avg), maximum HR (HR Max), and average HR (HR Avg), defined as follows:

10,000 foot-equivalent exposure
SpO_2 Min: minimum SpO_2 reached across all of S4.
SpO_2 Avg: mean SpO_2 for the last 15 min of S4.[3]
HR Max: maximum HR across all of S4.
HR Avg: mean HR for the last 15 min of S4.

25,000 foot-equivalent exposure
SpO_2 Min: minimum SpO_2 reached across all of S2 and S3.[4]
SpO_2 Avg: mean SpO_2 in the 30 s interval [tmin-29, tmin], where tmin = time SpO_2 Min occurred.
HR Max: maximum HR reached across all of S2 and S3.
HR Avg: mean HR in the 30 s interval [tmax-29,tmax], where tmax = time at which HR Max occurred.

[3] Participants' SpO_2 and HR typically required around 15 minutes to stabilize during exposure to the 10,000 foot-equivalent altitude. We therefore averaged over the last 15 minutes to capture a more stable estimate.
[4] S3 was included because there is a lag between the end of the hypoxic exposure and cessation of the downward trend in vital signs. Extreme values of SpO_2 and HR commonly occurred during the 25,000 foot-equivalent recovery segment after return to normal breathing oxygen levels.

Analyses

We conducted a series of repeated measures ANOVAs followed by planned comparisons to test for differences in FSE, LPM, and TSD in segments of primary interest (S2 and S4) across the different exposure profiles. Two-tailed tests with α set at 0.05 were used for all analyses, unless otherwise noted.

Exposure profiles will be referred to by letter designation only in this section. Set notation will be used when two or more profiles are considered together in planned comparisons. Outliers were identified according to the Tukey hinges method. Mean replacement and/or subject exclusion are noted below, identified in the format participant(profile) (i.e., 1(A) means participant 1, profile A).

Measure validation

We first confirmed that our measures were sensitive to the effects of hypoxia. We compared performance measures from S2 in A and B (ground level) to performance measures from S2 in C and D (25,000 feet equivalent). Because most participants did not reach the time limit during the 25,000 foot simulated exposure, interval S2 was typically shorter in conditions C and D. FSE and LPM were not biased by this difference due to standardization of these measures (described above). TSD was not standardized. The bias in this measure was negligible, however, as the shortest exposure still contained approximately 1200 samples (resulting in a correction factor on the order of 0.0001).

Planned comparisons for the 25,000 foot repeated measures ANOVA were as follows: {A, B} vs {C, D}; A vs B; C vs D. The primary comparison of interest to this study is the first. Worse performance in {C, D} compared to {A, B} would indicate that performance during the 25,000 foot simulated exposure was significantly worse than performance at ground level,

supporting the validity of the performance outcome measures (Figure 3). We ran the {A, B} vs {C, D} comparison as a one-tailed test because we expected performance during exposure to 25,000 feet to be worse than performance during exposure to ground level. The comparisons A vs B and C vs D were performed to check for test-retest reliability in the outcome measures. We did not expect to see differences in these secondary comparisons. Table 1 lists means and SD for each outcome measure during S2 of each profile.

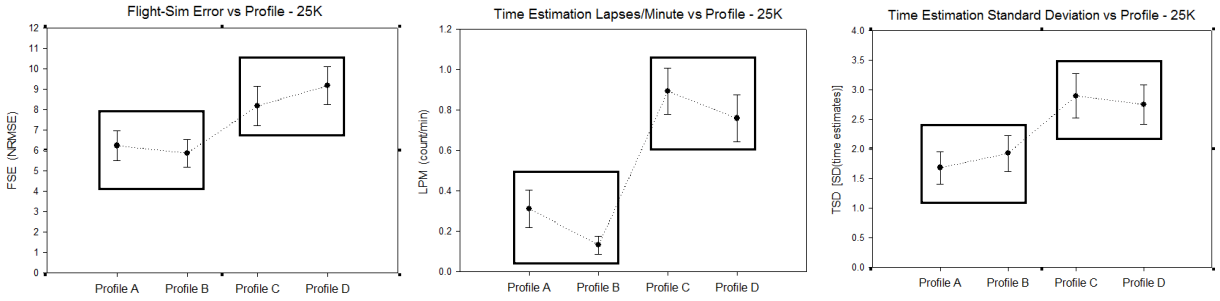

Figure 3. {A, B} to {C, D} comparisons for S2 across the three performance measures.

Measure	Profile A	Profile B	Profile C	Profile D	N
FSE	6.23 ± 3.23	5.87 ± 3.01	8.19 ± 4.33	9.18 ± 4.11	20
LPM	0.31 ± 0.42	0.13 ± 0.20	0.89 ± 0.51	0.76 ± 0.52	20
TSD	1.68 ± 1.22	1.93 ± 1.35	2.90 ± 1.69	2.75 ± 1.50	20

Table 1. Means and SD for each performance measure during S2 of each exposure profile.

Mean replacement was used for two subjects in the FSE analysis [13 (A, B, C) and 21 (A, B)], and two subjects in the LPM analysis [4(D) and 38(C, D)]. Mathematically, 4(D) and 38(D) were not outliers, but these participants appeared not to have understood the time estimation task for initial experiment runs (note that mean replacement in this instance guards against finding a significant difference based on spurious data). Mean replacement was used for 4(D) in the TSD analysis due to having 100% lapses during S2.

FSE was significantly different between profiles overall, $F(3, 57) = 4.97$, $p < 0.01$, $\eta_p^2 = 0.21$. As expected, planned comparisons revealed that FSE was greater in {C, D} compared to {A, B}, $F(1, 19) = 11.25$, $p < 0.01$, $\eta_p^2 = 0.37$. Planned comparisons also confirmed the expected non-significant differences between A and B, $F(1,19) = 0.30$, $p = 0.59$, and between C and D, $F(1, 19) = 0.73$, $p = 0.40$.

LPM was significantly different between profiles overall, $F(3, 57) = 15.26$, $p < 0.001$, $\eta_p^2 = 0.45$. Planned comparisons revealed that LPM was greater in {C, D} compared to {A, B}, $F(1, 19) = 70.42$, $p < 0.001$, $\eta_p^2 = 0.79$. Planned comparisons again revealed no differences between A and B, $F(1, 19) = 3.35$, $p = 0.08$, or between C and D, $F(1, 19) = 0.58$, $p = 0.46$.

TSD followed the same pattern of results as FSE and LPM. TSD was significantly different between profiles overall, $F(3,57) = 4.71$, $p < 0.01$, $\eta_p^2 = 0.20$. Planned comparisons revealed that TSD was greater in {C, D} compared to {A, B}, $F(1,19) = 14.03$, $p < 0.001$, $\eta_p^2 = 0.43$, and that no significant differences existed between A and B, $F(1, 19) = 0.64$, $p = 0.43$, and between C and D, $F(1, 19) = 0.10$, $p = 0.76$.

10,000 foot effects

For the 10,000 foot-equivalent exposure repeated measures ANOVA, we compared performance measures within S4 across conditions A, B, C, and D. S4 in A was the control condition, S4 in B and D was the actual 10,000 foot simulated exposure, and S4 in C was the 30 min timespan at ground level starting 5 minutes after the end of the 25,000 foot-equivalent exposure.

Planned comparisons for the 10,000 foot-equivalent exposure repeated measures ANOVA were as follows: A vs {B, C, D}; C vs {B, D}; B vs D. The comparison of primary interest to this study was B vs D. If performance during the 10,000 foot-equivalent exposure was

worse in D compared to B, an effect due to combined exposures is indicated (Figure 4). Due to the directional and a-priori nature of our hypotheses, we used one-tailed tests for the B vs. D comparisons for each outcome measure.

A difference between B and D does not by itself indicate that a combined effect occurred. Such a difference may be due to failure to recover from the original moderate exposure. In order to attribute the effect to combined exposures solely, we must examine whether performance is worse in D compared to C and in C compared to B. This could not be done simultaneously with planned comparisons. Hence, we used post hoc comparisons (with Sidak correction) to compare D to C and C to B. If performance during S4 in condition C is significantly worse than performance in condition B, but not different than condition D, a failure to recover from the moderate exposure is indicated. If performance in condition D was worse than condition C, a combined effect is indicated. If condition C is not statistically different than performance in either B or D, then we will be unable to distinguish between the two explanations statistically.

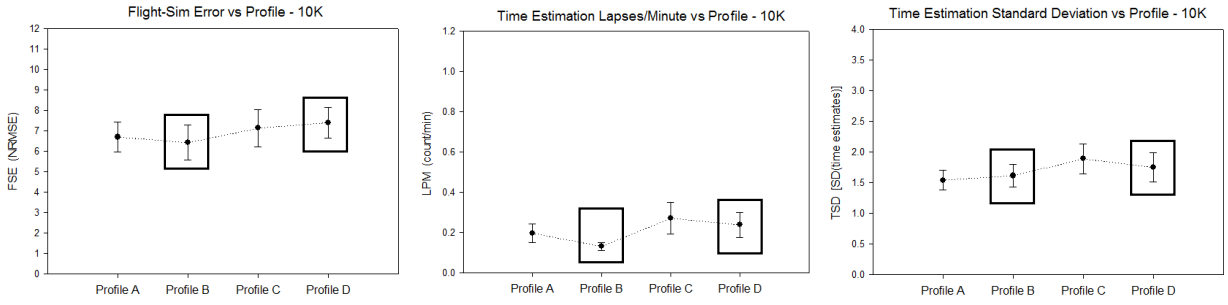

Figure 4. B vs D comparisons for S4 across each performance measure.

No participants were advanced during the 10K exposure; hence, all segments were 30 minutes long. Mean replacement was used for two participants in the FSE analysis [13(A) and 18(B)]. For LPM, mean replacement was used for 38(C, D) due to reasons given above. Two participants were outliers across all profiles for LPM (15 and 16); these subjects were excluded

from LPM and TSD analyses. There were no outliers for TSD. Table 2 lists means and SD for each outcome measure during S4 of each exposure profile.

Measure	Profile A	Profile B	Profile C	Profile D	N
FSE	6.70 ± 3.32	6.42 ± 3.82	7.13 ± 4.04	7.40 ± 3.32	20
LPM	0.20 ± 0.19	0.13 ± 0.08	0.27 ± 0.33	0.24 ± 0.27	18
TSD	1.54 ± 0.67	1.61 ± 0.79	1.89 ± 1.05	1.75 ± 1.00	18

Table 2. Means and SD for each performance measures during S4 of each exposure profile.

FSE was not significantly different between profiles overall, $F(3, 57) = 0.95$, $p = 0.43$. Planned comparisons revealed non-significant differences between A and {B, C, D}, $F(1, 19) = 0.20$, $p = 0.66$, and between C and {B, D}, $F(1, 19) = 0.26$, $p = 0.62$. Given a one-tailed α-level of 0.05, the difference between B and D came close to reaching significance, $F(1, 19) = 2.81$, $p = 0.06$, $\eta_p^2 = 0.13$. However, post hoc comparison (with Sidak correction) revealed no significant difference in FSE between D and C ($p = 1.00$) or C and B ($p = 0.74$).

LPM was not significantly different between profiles overall, $F(1.88, 31.95) = 1.66$, $p = 0.21$, with Greenhouse-Geisser correction. Planned comparisons revealed no significant differences between A and {B, C, D}, $F(1, 17) = 0.09$, $p = 0.77$, and between C and {B, D}, $F(1, 17) = 2.23$, $p = 0.15$. The difference between B and D was significant, $F(1, 17) = 2.94$, $p = .05$ (one-tailed), $\eta_p^2 = .15$. Post hoc comparison (with Sidak correction) again revealed no significant difference in LPM between D and C ($p = 0.99$), or C and B ($p = 0.42$).

TSD was not significantly different between profiles overall, $F(3, 51) = 1.62$, $p = 0.20$. Planned comparisons revealed no significant difference between A and {B, C, D}, $F(1, 17) = 1.57$, $p = 0.23$, or between C and {B, D}, $F(1, 17) = 2.24$, $p = 0.15$. In this case, the difference between B and D did not come close to reaching significance, $F(1, 17) = 0.94$, $p = 0.17$ (one-

tailed). Once again, post hoc comparison (with Sidak correction) revealed no significant difference between D and C ($p = 0.96$) or C and B ($p = 0.37$).

Physiological Measure Results

A series of paired t-tests was performed to test for differences in physiological measures between B and D during the 10,000 foot-equivalent exposure (S4), and between C and D during the 25,000 foot-equivalent exposure (S2) and recovery (S3). Table 3 displays means and SD during S4 along with *p* values for the physiological comparisons. We found no significant differences within S4 between conditions A and C, but we did find significant differences within S4 among all four physiological measures between conditions C and D. A small (MD = 1.74) but significant difference in SpO2 Min was found between B and D in segment S4, $t(19) = 1.73$, $p = .04$ (one-tailed).

Measure	Segment	Profile A	Profile B	Profile C	Profile D	*p*-value (A vs C)	*p*-value (B vs D)	*p*-value (C vs D)
SpO2 Avg	S4	99.31 ± 1.05	90.91 ± 2.45	99.59 ± 1.06	90.06 ± 2.77	0.39	0.13	< 0.01
SpO2 Min		97.57 ± 1.34	86.61 ± 2.47	97.78 ± 1.04	84.87 ± 4.37	0.47	0.04	< 0.01
HR Avg		74.57 ± 9.47	80.57 ± 11.91	73.71 ± 9.91	78.92 ± 10.14	0.63	0.18	0.01
HR Max		88.77 ± 11.87	93.26 ± 13.94	87.23 ± 12.09	92.27 ± 10.92	0.47	0.31	0.01

Table 3. Means and SDs during S4 and p values for physiological comparisons across conditions.

Recovery

We examined performance during the recovery flight one hour after exposure (R1) using similar methods as above. We conducted a repeated measures ANOVA for each outcome measure comparing performance during R1 across the four exposure profiles. We then used the following planned comparisons to compare performance between specific profiles (A vs {B, C, D}; B vs {C, D}; C vs D). Mean replacement was used for 13(A,B,C,D) and 16(C,D) in the FSE

analysis. Participants 15 and 16 were excluded from LPM and TSD analyses for consistency with previous ANOVAs. Mean replacement was used for 2(A) and 38(C,D) in the LPM analysis, and 13(D) in the TSD analysis. As we did not expect to find significant differences during the recovery periods, we used two-tailed significance tests with α set at 0.05. Means for each outcome measure in each condition are found in Table 4.

Measure	Profile A	Profile B	Profile C	Profile D
FSE	4.64 ± 2.09	4.43 ± 1.69	5.06 ± 2.65	4.59 ± 1.63
LPM	0.12 ± 0.14	0.05 ± 0.09	0.09 ± 0.12	0.10 ± 0.14
TSD	1.47 ± 0.97	1.16 ± 0.48	1.47 ± 0.85	1.13 ± 0.61

Table 4. Means and SD for each condition during the one hour recovery flight

FSE was not significantly different between profiles overall, $F(3, 57) = 0.73$, $p = 0.54$. Planned comparisons revealed no significant differences between A and {B, C, D}, $F(1, 19) = 0.02$, $p = 0.88$, between B and {C, D}, $F(1, 19) = 1.76$, $p = 0.21$, and between C and D, $F(1, 19) = 0.74$, $p = 0.40$.

LPM did not demonstrate significant differences between profiles overall, $F(1, 51) = 1.03$, $p = 0.39$. Planned comparisons revealed no significant differences between A and {B, C, D}, $F(1, 17) = 1.29$, $p = 0.27$, between B and {C, D}, $F(1, 17) = 2.13$, $p = .16$, and between C and D, $F(1, 17) = 0.08$, $p = 0.78$.

TSD was not significantly different between profiles overall, $F(1.79, 30.49) = 1.86$, $p = 0.18$ (with Greenhouse-Geisser correction). Planned comparisons revealed no significant differences between A and {B, C, D}, $F(1, 17) = 0.90$, $p = 0.36$, between B and {C, D}, $F(1, 17) = 1.96$, $p = 0.18$. However, a significant difference was found between C and D, $F(1, 17) = 5.33$, $p = 0.03$. Conditions A and B demonstrated a similar pattern as conditions C and D, but post-hoc

comparison (with Sidak correction) revealed no significant difference between A and B (p = 0.72).

Analyses for R2 revealed no significant differences across conditions for any of the performance measures. Physiological measures had all returned to baseline by the end of the original hypoxic exposure; we therefore did not examine physiological measures for either of the recovery sessions.

Discussion

Summary

In contrast to most research examining performance at different altitudes in isolation, this study examined performance under conditions of sequential exposures such as those that may occur during an in-flight hypoxic event. Conventional wisdom holds that performance recovers with SpO_2 following a hypoxic exposure and that supplemental oxygen is not necessary at altitudes below 10,000 feet. The findings of this study indicate that at least one of these assumptions may not be true under the conditions encountered during an in-flight hypoxic emergency. Performance on a simulated flight task and time estimation task indicated that performance may not recover immediately after a hypoxic exposure. SpO_2 data indicated that the physiological effects of exposure to 10,000 feet simulated altitude may be exacerbated by prior exposure to moderate hypoxia.

Discussion of the performance measures

When comparing S4 across conditions B and D, we found evidence that FSE and LPM increased in condition D compared to condition B (although LPM did not quite attain significance). This indicates that performance during a 10,000 foot simulated exposure was worse when preceded by exposure to a 25,000 foot simulated altitude than when the exposure

occurred in isolation. Two hypotheses may explain this pattern of results. The first is that the moderate and mild exposures interact such that the effects of the first exposure linger to increase the effects of the second exposure. The other is that the effects of the first exposure never fully dissipated upon return to normal SpO_2, and we have simply identified a failure to recover from the first moderate hypoxic exposure rather than an interaction between multiple exposures. We examined these hypotheses by comparing S4 in conditions B, C, and D.

Post-hoc tests comparing S4 in condition C to conditions B and D across all three performance measures indicated no difference between these conditions. This result makes it impossible for us to determine statistically which explanation is more likely based on our data. However, the means for S4 in condition C across both of the time estimation tasks indicated *worse* performance than condition D (see Figure 4). Given the very conservative nature of the post-hoc corrections, and the fact that the a priori test for LPM between conditions B and D demonstrated significance with a smaller mean difference, we believe that performance on our tasks in condition C is much more similar to condition D than condition B. Further, there is no reason to expect such a pattern of means in the context of an additive effect across the exposures (i.e., performance should not be better in condition D than condition C if there is any added performance decrement at 10,000 feet simulated altitude). We are therefore inclined to believe that the observed performance effects are due to a failure to recover completely following exposure to moderate hypoxia rather than an interaction between the two exposures.

Discussion of the physiological measures

Pairwise tests comparing S4 across the different exposure profiles indicated that vital signs returned to normal within minutes after exposure to moderate hypoxia when recovering at ground level, but not when recovering from moderate hypoxia at 10,000 feet simulated altitude.

This finding is to be expected given the difference between ground level and 10,000 feet, and the SpO_2 and HR observed at 10,000 feet simulated altitude during condition D were not in the range that would cause concern. However, we did observe a small but statistically significant difference in SpO_2 Min between conditions B and D during S4. Participants reached a lower minimum SpO_2 when breathing at 10,000 feet simulated altitude if they had previously been exposed to 25,000 feet simulated altitude.

This result indicates that some physiological interaction between the two hypoxic exposures may have occurred. However, SpO_2 data does not necessarily correspond to performance. Prior research in our lab has demonstrated continued impairment after hypoxic exposure despite returning to normal SpO_2 levels (Phillips et al., 2009; Phillips, Simmons, & Horning, 2012). The similarity in performance despite vastly different SpO_2 levels during S4 seen between conditions C and D in this study further supports such a claim. We therefore believe that something other than change in SpO_2 is driving the performance effects seen in this study.

Discussion of the follow up sessions

We did find one significant difference during the recovery flights such that condition C appeared to show worse performance than condition D on the TSD measure one hour after exposure. This was a surprising result because it does not fit with the pattern of results from the prior performance analyses and we do not have a ready explanation for the direction of the difference. Prior analyses showed no difference between conditions C and D during exposure; finding a difference one hour later is counterintuitive. Had condition D been worse than condition C one hour later, an interactive effect between hypoxic exposures may have been implied. However, the opposite effect was found. The analysis indicated that TSD in condition C

was significantly worse than condition D one hour after hypoxic exposure. An examination of the means during sea level exposures implied that the difference was because participants actually improved relative to baseline in condition D. Conditions A and B showed a similar pattern such that people appeared to improve after breathing 10,000-foot equivalent oxygen mixtures. Post-hoc tests lacked sufficient power to detect a difference between these conditions, but we strongly suspect that an a priori test would have been significant given the similar mean differences.

Other than some type of rebound effect from low-level hypoxic exposure, we can think of no theoretical or physiological explanation to account for why a person would improve one hour after breathing 10,000 feet-equivalent oxygen levels instead of normal oxygen levels. Given the lack of a theoretical or physiological explanation for this finding and the fact that we only observed these effects on one performance measure, we are tempted to explain this finding as a spurious result. However, the apparent consistency of the effect between conditions {A, B} and {C, D} makes us hesitate to conclude definitively that the effect is not "real". At this time we can only report the finding and note that it does not appear to fit with any previously observed pattern of results. Any possible effects appear to have dissipated two hours after exposure, however.

Implications

Our assumptions about how well pilots are able to recover after a hypoxic exposure may be incorrect. Current emergency procedures in response to an in-flight hypoxic event prescribe that pilots breathe a limited supply of supplemental oxygen to recover while descending to a cabin altitude of 10,000 feet, at which point they remove the breathing mask and breathe cabin air. Our data indicate that people do not recover immediately after exposure to moderate

hypoxia, and that pilot performance while recovering at 10,000 feet after such an exposure may therefore remain impaired to some degree.

Limitations

One of the main limitations that complicated interpretation of our results was the fact that our counterbalancing scheme was altered due to participant dropout. Uneven dropout among participants across condition presentation order caused condition A to be presented first for eight of the 21 participants who completed the study (compared to four, five, and four for conditions B, C, and D, respectively). Condition presentation order was therefore confounded with experience in the simulator, possibly leading to worse performance during the control condition despite the training time given to participants.

Time-on-task effects may have compounded this issue. We sought to replicate the types of exposures that would be likely in an emergency situation, requiring a relatively long period of exposure and data collection. Participants likely became somewhat bored during the later portions of the exposure, particularly in condition A when they lacked the additional challenge of a hypoxic exposure.[5] These two causes together are likely what caused performance in condition A to appear slightly worse than condition B in Figures 3 and 4, as we can think of no physiological reason that exposure to 10,000 feet simulated altitude should improve performance relative to ground level. The fact that our control condition was not our "best" condition in terms of performance made it difficult to use condition A as a baseline to determine the performance effects of conditions B and C alone. However, we do not believe this issue affected our primary results or interpretation in a substantive way.

[5] In spite of our efforts to keep participants blinded regarding the exposures, most people are able to determine whether they are experiencing hypoxia due to symptomology and changes in the ROBD-2. This is particularly true if participants have prior experience with hypoxia.

A second limitation of this study concerns the sensitivity of our performance measures. We sought to balance the sensitivity of our measures, the realism of the task, and practicality. As a result, we did not use the most sensitive performance measures known. It is possible that a more sensitive measure such as reaction time may have demonstrated stronger results. However, we felt it was important to use performance measures with direct relevance to the flight environment, and we were further concerned that time-on-task effects would be exacerbated if participants were asked to perform a less engaging reaction time task for the 50 minute duration of the exposure.

The third limitation of this study is a lack of fidelity in the recovery procedure. Participants in our study recovered by breathing ground level oxygen concentrations. However, pilots in an emergency situation breathe 100% oxygen after the initial exposure. We did not use 100% oxygen in order to avoid a possible confound in our results. Anecdotal evidence from training and unpublished data from our lab indicates a possibility that breathing 100% oxygen may temporarily exacerbate symptoms in a phenomenon termed the "oxygen paradox". This study is the first to examine possible interactions between moderate and mild hypoxic exposures and we wanted to ensure that any possible performance deficits were due to the hypoxic exposure rather than the recovery procedure.

Finally, we did not use licensed pilots in this study due to practical concerns about our ability to recruit sufficient numbers of participants and a previous history of using non-pilots in the simulator successfully. As a result, we were somewhat limited in the flight tasks that we could use. For example, approach and landing tasks are particularly sensitive to mild hypoxia (Nesthus, Rush, & Wreggit, 1997), but we could not utilize these more complicated flight tasks with a non-pilot population. Despite this limitation, however, we were able to demonstrate

performance effects during moderate hypoxia and mild hypoxia with a simple flight and secondary task. We would argue that if one cannot maintain straight and level flight in conjunction with a simple secondary task, one cannot expect to pilot an aircraft through more complicated maneuvers while simultaneously navigating and communicating with air traffic controllers.

Recommendations for future research

Our data strongly implies that performance deficits may persist beyond the period of hypoxic exposure, even after heart rate and SpO_2 return to normal. However, this effect was inconsistent across our performance measures. Prior research has demonstrated similar results, but the effect has likewise been inconsistent across tasks (Phillips et al., 2009; Phillips, Simmons, & Horning, 2012). Further work is needed to determine what aspects of performance are likely to show continued impairment after a hypoxic event. We must then replicate this effect using more sensitive measures of these aspects of performance in order to better understand the ways in which pilots are likely to remain impaired following an in-flight emergency.

As mentioned above, this study used ground level oxygen concentrations after the exposures rather than 100% oxygen. Previous work has indicated that 100% oxygen can reduce recovery time after a hypoxic exposure, but not for every task (Phillips et al., 2015; Phillips et al., 2016). Future work should investigate whether 100% oxygen leads to faster recovery and reduces the deficits observed in this study.

While the performance data indicated that our findings are most likely the result of a failure to recover from the original moderate exposure, the physiological data indicated that a small additive effect of the moderate and mild exposures on SpO_2 was possible. Future work should examine these two explanations to try to tease apart the mechanisms of prolonged

impairment following a hypoxic exposure in the context of current emergency procedures. If multiple exposures do in fact have an additive effect, emergency procedures may need to be modified to minimize this impact.

Finally, we noted an unexpected result indicating that performance may improve one hour after breathing 10,000 feet normobaric equivalent oxygen levels relative to ground level. Because we are not able to judge the validity of this result in the present study, future work should try to replicate this effect. If confirmed, such a finding would be very interesting and we should work to ascertain any potential causal mechanisms.

Conclusions

The data in this study indicate that at least one of the assumptions underlying current emergency procedures may not hold true. We found that performance may be impaired during a mild exposure to hypoxia when preceded by a moderate hypoxic exposure. Based on prior research findings and the patterns seen in our own data, we believe this effect is due to a failure to recover from the moderate exposure. However, we are currently unable to rule out the possibility of an additive effect between the two exposures with 100% certainty. Regardless of the cause, the findings of this study challenge the notion that pilots are able to recover to baseline after descending following a hypoxic emergency. More work is needed to ascertain the precise nature of hypoxia's effect on performance in order to better predict what aspects of performance are likely to remain impaired following an in-flight emergency. If performance remains significantly impaired following exposure to hypoxia, more focus should be turned to preventing exposure via in-cockpit sensors to proactively alert the pilot before physiology is affected.

References

Artino, A. R., Folga, R. V., & Swan, B. D. (2006). Mask-on hypoxia training for tactical jet aviators: Evaluation of an alternative instructional paradigm. *Aviation, Space and Environmental Medicine, 77,* 857-863.

Bortolussi, M., Hart, S., & Shively, R. (1989). Measuring moment-to-moment pilot workload using synchronous presentations of secondary tasks in a motion-based trainer. *Aviation, Space, and Environmental Medicine, 60,* 124-129.

Bortolussi, M., Kantowitz, B., & Hart, S. (1986). Measuring pilot workload in a motion base trainer: A comparison of four techniques. *Applied Ergonomics, 17.4,* 278-283.

Casali, J., & Wierwille, W. (1983). A comparison of rating scale, secondary-task, physiological, and primary-task workload estimation techniques in a simulated flight task emphasizing communications load. *Human Factors, 25,* 623-641.

Connolly, D. M., Barbur, J. L., Hosking, S. L., & Moorhead, I. R. (2008). Mild hypoxia impairs chromatic sensitivity in the mesopic range. *Investigative Ophthalmology and Visual Sciences, 49,* 820-827.

DeHart, R. L. (Ed.). (1985). *Fundamentals of aerospace medicine*. Philadelphia, PA: Lea & Febiger.

Fowler, B., Banner, J., & Pogue, J. (1993). The slowing of visual processing by hypoxia. *Ergonomics, 36,* 727-735.

Fowler, B., Taylor, M., & Porlier, G. (1987). The effects of hypoxia on reaction time and movement time components of a perceptual-motor task. *Ergonomics, 30,* 1475-1485.

Fowler, B., White, P., Wright, G., & Ackles, K. (1982). The effects of hypoxia on serial response time. *Ergonomics, 25,* 189-201.

Krueger, G., Armstrong, R., & Cisco, R. (1985). Aviator performance in week-long extended flight operations in a helicopter simulator. *Behavior Research Methods, Instruments, & Computers, 17,* 68-74.

Legg, S., Hill, S., Gilbey, A., Raman, A., Schlader, Z., & Mundel, T. (2014). Effect of Mild Hypoxia on Working Memory, Complex Logical Reasoning, and Risk Judgment. *The International Journal of Aviation Psychology, 24*(2), 126-140.

Legg, S., Hill, S., Mundel, T., Gilbey, A., Schlader, Z., & Raman, A. (2012). Could mild hypoxia impair pilot decision making in emergencies? *Work, 41,* 198-203.

Nesthus. T., Rush, L. L., & Wreggit, S. S. (1997). *Effects of mild hypoxia on pilot performance at general aviation altitudes.* (Report No. DOT/FAA/AM-97-9). Federal Aviation Administration, Office of Aviation Medicine, *Washington DC.*

Petrassi, F., Hodkinson, P., Walters, P., & Gaydos, S. (2012). Hypoxic hypoxia at moderate altitudes: Review of the state of the science. *Aviation, Space, and Environmental Medicine, 83,* 975-984.

Phillips, J., Drummond, L., Robinson, F., Warner, S., VerStraten, M., & Funke, M. (2016, April). Differing oxygen concentrations and the effect on post-hypoxia recovery. Paper presented at the annual meeting of the Aerospace Medical Society, Atlantic City, NJ.

Phillips, J., Horning, D., Robinson, F.E., Warner, S., Gomez, J., Geyer, D., Drummond, L. et al. (2015). Recovery from hypoxic exposure. *Paper presented at the 85th Annual Aerospace Medicine Association Conference, Orlando, FL.*

Phillips JB, Simmons RG, Florian JP, Horning DS, Lojewski RA, Chandler JF. (2009). Moderate intermittent hypoxia: Effect on two-choice reaction time followed by a significant delay in recovery. *Proceedings of the Human Factors and Ergonomics Society 53rd Annual Meeting* (pp. 1564-1568). San Antonio, TX.

Phillips JB, Simmons RG, Horning DS (2012). Post-hypoxic recovery of cognitive and perceptual function. *Proceedings of the 83rd Annual Scientific Meeting of the Aerospace Medical Association*. Virginia: American Medical Association.

Previc, F., Lopez, N., Ercoline, W., Daluz, C., Workman, A., Evans, R., & Dillon, N. (2009). The effects of sleep deprivation on flight performance, instrument scanning, and physiological arousal in pilots. *Technical report #AFRL-HE-BR-JA-2006-0021.*

Smith, J., & Caldwell, J. (2004). Methodology for evaluating the simulator flight performance of pilots. *Technical report #AFRL-HE-BR-TR-2004-0188.*

Temme, L., Still, D., & Acromite, M. (2010). Hypoxia and flight performance of military instructor pilots in a flight simulator. *Aviation, Space, and Environmental Medicine, 81,* 654-659.

Appendix 1: Initial eligibility screening questionnaire

Participant #:_____ Date: _____

Gender: Male / Female Age: _____

Hand Dominance: Right / Left

Medical/Background screening

To the participant: Before we can schedule you for participation we need to ask a few questions about your background and medical history so that we can make sure that it's safe for you to be hypoxic. All information collected will be kept confidential.

1. Are you comfortable with a blood draw? YES NO
2. Do you have a recent history of living at altitude? (> 5000ft) YES NO

   If YES, how recently and for how long? _____
3. Have you ever been exposed to a hypoxic environment for research or in-flight? YES NO

   If YES, please explain (how long ago and why): _____
4. Are you in your usual state of fitness? YES NO

   If NO, please indicate the reason: _____
5. Do you currently have or have you ever been diagnosed with asthma? YES NO

   If YES, do you have normal pulmonary function? YES NO
6. Have you ever been diagnosed with heart/circulatory disease? YES NO
7. Do you currently have or have you ever been diagnosed with high blood pressure? YES NO
8. Have you ever been diagnosed with emphysema? YES NO
9. Have you ever been diagnosed with anemia? YES NO
10. Have you been diagnosed with epilepsy? YES NO
11. Have you ever tested positive for the sickle cell trait? YES NO
12. Have you had pneumonia within the last year? YES NO
13. Have you used tobacco products habitually within the last 6 months (more than 2 cigarettes per day)? YES NO

    If YES, please state frequency: _____
14. Do you have a history of fainting? YES NO
15. Have you donated blood or plasma in the past 30 days? YES NO

16. Are you taking any prescribed medication on a regular basis, or a temporarily prescribed medication, within the past 7 days? YES NO

    If YES, please list: _____

17. Do you take any over-the-counter medications (e.g., antacids, YES NO

    Benadryl, Tylenol,) on a regular basis (2 or more times a month)?

    If YES, please list: _____

18. Do you take an herbal, protein, or power enhancing supplement

    on a regular basis? YES NO

    If YES, please list: _____

19. How many alcoholic beverages do you consume per day on average? _____

20. Are you claustrophobic? YES NO

21. Can you think of anything else regarding your history or present physical state which might affect your performance?

Appendix 2: Participant compliance questionnaire

Participant #:_____ Date: _____

1. Have you donated blood or plasma since screening (or your most recent visit)? YES NO
2. Have you used tobacco products since screening (or your most recent visit)? YES NO
3. Have you been ill in the past week? YES NO

   If YES, please indicate:

   1. The nature of the illness (flu, cold, etc.): _____
   2. Severity of the illness: Very 1 2 3 4 5 6 7 8 9 Very
      Mild Severe
   3. Length of illness: ____Hours ____Days
   4. Major symptoms: _____
   5. Are you fully recovered? YES NO

4. Have you consumed any caffeine within the past 48 hours? YES NO
   a. If yes, how much? _____
   b. Is this your normal amount? _____
5. Have you consumed any alcohol within the past 48 hours? YES NO
   a. If yes, how many drinks? _____
6. Have you been above 5,000 feet since screening (or your most recent visit)? YES NO
7. Have you taken any supplements in the last 48 hours? YES NO
   a. If yes, please list _____
8. Have you taken any over-the-counter medications in the last 48 hours? YES NO
   a. If yes, please list _____
9. Have you taken any prescription medication in the last 48 hours? YES NO
   a. If yes, please list _____
10. How many hours of sleep did you get last night? _____Hours
    a. Was this amount sufficient? YES NO
    b. Is this your normal amount? YES NO